PHARMACOLOGICAL EVALUATION OF *CANTHIUM COROMANDELICUM (Burm.f)Alston* IN EXPERIMENTAL ANIMAL MODELS

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In partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

By

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CERTIFICATE

This is to certify that the investigation described in this dissertation entitled **PHARMACOLOGICAL EVALUATION OF *CANTHIUM COROMANDELICUM* (Burm.f) Alston IN EXPERIMENTAL ANIMAL MODELS** Submitted by Reg No: (561225352) was carried out in the department of Pharmacology, Arulmigu Kalsalingam College of Pharmacy, Anand Nagar Krishnan koil- 626 126, which is affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai, Under the guidance of Dr.P.Thirupathy Kumaresan, M.Pharm, Ph.D., Professor, Department of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil.

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EVALUATION CERTIFICATE

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Examiners

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LIST OF ABBREVIATIONS USED

b.w.	-	Body weight.
CPCSEA	-	Committee for the purpose of control and supervision of experiments on animals.
ext.	-	Extract.
fig	-	Figure.
g m	-	Gram.
Mg	-	Milli gram
ml	-	Milli litre
IAEC	-	Institutional Animal Ethics Committee.
P.O.	-	Per oral .
LD50	-	Lethal dose.
SGOT	-	Serum glutamate oxalo acetae transferace
SGPT	-	Serum glutamate pyruvate transferace.
No	-	Number .
ANOVA	-	Analysis of variance .
SEM	-	Standard error mean.
Con	-	concentration.
MECC	-	Methanol extract of canthium coromandelicum
AECC	-	Aqueous extract of canthium coromandelicum
CECC	-	Chloroform extract of canthium coromandelicum
PECC	-	Petroleum ether extract of canthium coromandelicum
TLC	-	Thin layer chromatography

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CHAPTER 1

INTRODUCTION

Man's existence on this earth has been made possible only because of the vital role played by plant kingdom. Nature always stands as golden mark to amplify the outstanding phenomenon of symbiosis. Medicinal plants existing even before human being made their appearance on the earth[1].

Traditional medicine using herbal drugs exists in every part of the world. The major areas are Chinese, Indian and European traditions. The philosophies of these traditional medicines have some resemblance to each other but differ widely from modern western medicine. In view of the progress of western medicine not only new synthetic drugs but also herbal drugs have to fulfill the international requirements on quality, safety and efficacy. Herbal drugs have the advantage of being available for patients in the geographical area of the special traditional medicine. The development procedure of herbal drugs for world-wide use has to be different from that of synthetic drugs[2].

Practically every country develops its own medical system, which includes the ancient civilization of China, Egypt and India. Thus, the Indian medical System Ayurveda came into existence. The raw materials for ayurvedic medicines were mostly obtained from plant sources in the form of crude drugs such as dried herbal powders or their extracts or mixture of products[3]. Also, Siddha, Unani and Tibb are traditional health care systems which have been flourishing for many centuries. Apart from these systems there has been a rich heritage of ethnobotanical usage of herbs by various colorful tribal communities in the country[4].

In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded across the countries. Therefore the use and history of herbs dates back to the time of early man, who had the crudest tools as his implement and use stones to start his fire. They used herbs in their raw and cooked forms to keep fit. Since that time , the use of herbs has been known and accepted by all nations and has been known as the first art of treatment available to man.

Plants are the only economic source of a number of well established and important drugs. In addition they are the source of some chemical intermediates needed for the production of a number of drugs. Natural production are an integral part of human health care system now-a-days because there is now popular concern over toxicity and side effects of modern drug. There has been regurgence in the consumption and demand for medicinal plants, these plants are finding use as pharmaceuticals, Neutrachemicals, cosmetics and food supplements. Even as a traditional sources of medicine they continue to play a vital role.

Interest in medicinal plants has increased enormously over the last two decades. The entrapped wealth of the plant kingdom has become a target for the search of multi national drug companies and research institute for new drugs and lead compounds. There is no plant that does not have medicinal value. The active components are normally extracted from all plants structures but the concentration of these compounds vary from structure to structure, however parts known to contain the highest concentration of the principle are preferred to leaves, stem, barks, roots, bulks, corms, rhizomes, woods, flowers, fruitsor the seeds[5].

Vast ethnobotanical knowledge exists in India from ancient time. Our work over four decades, both in the field and literary studies, has resulted in a dictionary of Indian folk-medicine and ethnobotany that includes 2532 plants. India has about 45,000 plant species; medicinal properties have been assigned to several thousand. About 2000 figure frequently in the literature; indigenous systems commonly employ 500. Despite early (4500-1500 BC) origins and a long history of usage, in the last two centuries, Ayurveda has received little official support and hence less attention from good medical practitioners and researchers. Much work is now being done on the botany, pharmacognosy, chemistry, pharmacology and biotechnology of herbal drugs. The value of ethnomedicine has been realized; work is being done on psychoactive plants, household remedies and plants sold by street drug vendors.

India unquestionably occupies the top position in the use of herbal drugs. It is one of the foremost countries exporting plant drugs or their derivatives and excels in home consumption too. According to Indian mythology, when the illness and disease got rampant on the earth, the sages learnt the science of healing from Lord Indra and recorded them in scriptures[6].

It has been estimated that about 75,000 species of higher plants exist on the earth. A reasonable estimate of about 10% has been used in traditional medicine. However, perhaps only about 1% of these are acknowledged through scientific studies to have therapeutic value when used in extract form by human[7].

Traditional healers and pharmacists in developing countries are in important source of information about plant sources of new drugs. Only a fraction of the earth's natural pharmacopoeia has been analyzed with modern techniques. The threat of imminent extinction of many plant species, especially in tropical areas, makes it urgent that scientists learn as much as possible before old remedies are forgotten or their raw materials are destroyed. This process requires the observation and recording of medical techniques, identification of plant materials and experimental investigation of the ingredients and their effects. Ethnopharmacology can also be an important element of a developing nation's medical and economic system. Third World governments are being encouraged to seek a synthesis between modern and traditional medicine. Although developing countries are providing many of the raw materials needed in drug manufacturing, the final products are often returned as high-priced medicines. As more plants are needed for large-scale production, over harvesting has led to stock depletion. Chemists have so far been unable to reproduce the complex structure of many plant compounds. further coordinated research into folk traditions, plant species, growing conditions and local medical needs is urged. care must be taken however to preserve the main advantages of traditional medical care, low cost and easy access[8].

Herbal Wealth of India [9]

Now-a-days natural products are an integral part of human health care system, because there is popular concern over toxicity and resistance of modern drugs. India is one of the 12 leading biodiversity centers with presence of over 45,000 different plant species, 15000-18000 flowering plants, 23,000 fungi, 16,000 lichens, 18,000 bryophytes and 13 million marine organisms. From this flora, 15,000 to 20,000 have good medicinal value. Among those only about 7,000 plants are used in Ayurveda, 600 in Siddha, 700 in Unani and 30 in modern medicines.

Researches in isolated plant constituents are of greater importance, it has given rise to many of the world's most useful drugs. Tubocurarine, the most powerful muscle relaxant, in existence is derived from curare and the strongest pain killer of all, morphine comes from poppy cocaine from coca.[10]

Diseases always co existed with livings, detecting their remedies also always continuing, going through the commencement of drug therapy for disease, drug comes to to the force in sudden, in the ancient time human knowledge found the absence of some forms the base for the development of some disease, they were tried to use the particular disease and they got success in that work. This motivate the plant researches to use different plants, plant parts for different disease.

Our traditional system of medicines siddha categorized nearly 5000 plants species and their usage. Later on the allopathic system of medicine comes to force and dominate the siddha and due to the fast relieving nature it reaches the world as quickly and diminished the usage of plant medicine as maximum.

But allopathy system cannot provide ultimate solution to some diseases, and also their side effect in particularly the long term therapy, limits their usage still plant medicine is recommended and usage still the plant medicine is recommended and used in such cases. This suggests the plant medicine to researches as and scientific world as alternate to allopathy system of medicine. The world health organization also recognize and motivate the plant researches and centre, hence the plant medicine now considered to be alternative system of medicine.

Even usage of plants are known since plants species consist of mixture of compound, isolating the single compound and identifying the component is responsible for that particular activity is a major question in front of plant researches and also it is very difficult to say only these are all the compounds available from particular plant.

Now a days due to the development of science and technology such as chromatography technique and spectroscopical technique it is possible to isolate almost all the components of plant and characterize them. Isolating and characterization are very important to improve effectiveness, minimizing the dose and on set of action.

REVIEW OF LITERATURE

LIVER

Liver is the heaviest gland of the body weighing about 1.4 kg in an average adult and is inferior to the diaphragm occupying most of the right hypochondriac and a part of the epigastric region of abdominopelvic cavity. The liver is divided into right and left lobe constituted by hepatocytes which are arranged in irregular, branching interconnected plates around a central vein. The liver also consists of sinusoids through which the liver receives the blood. The sinusoids consists of fixed phagocytes called stellate reticuloendothelial (kupffer) cells which destroy the worn out white blood cells, red blood cells, bacterial and other foreign matter in the venous blood draining from the gastrointestinal tract. Bile is partially excretory product and partially a digestive secretion from hepatocytes. The sodium and potassium salts of bile acids play an important role in emulsification and breakdown of large lipid globules into a suspension of droplets and also in the absorption of lipids following their digestion[11].

The liver has an enormous task of maintaining the body‘s metabolic homeostasis. This includes, the processing of dietary amino acids, carbohydrates, lipids, and vitamins; synthesis of serum proteins; and detoxification and excretion into bile of endogenous waste products and pollutant xenobiotics. Hepatic disorders have far reaching consequences, given the critical dependence of other organs on the metabolic functions of the liver. Liver injury and its manifestations tend to follow characteristic patterns. In some instances, the diseased process is primary to the liver. In others, the hepatic involvement is secondary, often to some of the most common diseases in humans, such as cardiac decompensation, alcoholism and extrahepatic infections with progression of diffused disease or strategic disruption of circulation or bile flow.

In hepatic injury five general responses are seen, viz;

1) Inflammation: Injury to hepatocytes associated with an influx of acute or chronic inflammatory cells into the liver is termed hepatitis. Attack of viable antigen- expressing liver cells by sensitized T-cells is a common cause of liver damage. Inflammation may be limited to portal tract or may spill over into the parenchyma.

E.g., viral hepatitis due to hepatitis A virus (HAV), HBV, HCV, HDV and HEV.

2) Degeneration: The hepatocytes get damaged due to toxic or immunological insult and show an edematous appearance. Degeneration can also be in the form of stetosis, where in there is accumulation of fat droplets within the hepatocytes. E.g., hepatic degeneration can be due to genetic diseases or exogenous substance such as alcohol.

3) Cell death: Cell death which is toxic or immunologically mediated occurs via apoptosis wherein the hepatocytes become shrunken, pyknotic, and intensely eosinophilic. Alternatively, hepatocytes may also undergo lytic necrosis (osmotically swell and rupture). The other types are centrilobular necrosis, bridging necrosis, submassive necrosis and massive necrosis.

4) Fibrosis: Fibrotic tissue is formed in response to inflammation or direct toxic insult to the liver. Deposition of collagen has lasting consequences on hepatic pattern of blood flow and perfusion of heapatocytes. Initially fibrosis may develop within or around portal tracts or the central vein or may be deposited directly with in the sinusoids. Progressively, these fibrous strands link regions of the liver (portal-to- portal, portal-to-central, central-to-central), a process called bridging fibrosis. Fibrosis is generally considered as an irreversible consequence of hepatic damage.

5) Cirrhosis: Cirrhosis with continuing fibrosis and parenchymal injury, the liver is subdivided into nodules of degenerating hepatocytes surrounded by scar tissue,termed cirrhosis and is an end stage form of liver.

The clinical consequences of liver diseases are hepatic dysfunction in the form of jaundice, hypoalbuminemia, hyperammonemia, hyperglycemia, fector hepatitis, palmar erythema, spider angiomas, hypogonadism, gynecomastia, weight loss, muscle wasting, and portal hypertension from cirrhosis. If these are not treated promptly, they will lead to life threatening complications like hepatic failure in the form of hepatic encephalopathy, hepatorenal-syndrome; or portal hypertension from cirrhosis, Malignancy with chronic disease and hepatocellular carcinoma [12,13,14,15,16]

According to WHO about 18,000 people die every year due to liver diseases. The common ailments of liver are cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, fulminant hepatic failure and certain tumors like hepatoma. It is estimated that two billion people around the world are infected with hepatitis B. About 350 million of these have the chronic form of the disease. This alarming statistics with perplexing report, warrant the

immediate necessity of studies of any level to either ensure the effectiveness of available formulations or exploration of the new herbal therapies to reduce the morbidity and mortality rate due to hepatic complications. In modern medicine cortico steroids and immunosuppressant's are commonly used to treat liver disease in allopathic form of medicine. These drugs are associated with adverse effects such as immunosuppression and bone marrow depression. Further, the success rate of treating liver diseases is disappointing. Attempts are being made globally to get scientific evidences for this traditionally reported herbal drugs[17].

About 600 commercial preparations with claimed liver protecting activity are available all over the world. About 100 Indian medicinal plants belonging to 40 families are used for herbal formulation. A few reports on the hepatoprotective activity are cited here, e.g. *Apium graveolens Linn*. (Umbelliferae), *Boerhaaia diffusa* Linn. (Nyctagina ceae), *Euphorbia antisyphilitica* (Euphorbiaceae), *Rubia cordifolia* (Rubiaceae), *Solanum lyratum* (Solanaceae), *Tylophora indica* (asclepiadaceae)[18].

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated. In spite of phenomenal growth of modern medicine, there are few synthetic drugs available for the treatment of hepatic disorders. However there are several herbs claimed to have possessed beneficial activity in treating hepatic disorders[19]. But they need to be validated in the light of science to ensure their ability to conserve therapeutic effectiveness in the formulation form.

In spite of tremendous studies in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cells. However there are a number of drugs employed in traditional system of medicine for liver affections.

Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective action. However, numerous medicinal preparations have been advocated a traditional system of medicine, specially in ayurvedic, for treating liver disorders. Only a small proportion of hepatoprotective plants a s well as formulations used in traditional medicine are pharmacologically evaluated for their efficiency.

1.1 Anatomy of Liver: [20]

Liver is the largest gland of the body enclosed within the right lower rib cage beneath the diaphragm. It is almost completely covered by visceral peritoneum and a dense irregular connective tissue layer that lies deep to the peritoneum. Liver is divided in two principle lobes, a large right lobe and a smaller left lobe separated by falciform ligament. The right lobe is considered by many anatomists to include an inferior quadrate lobe and a posterior quadrate lobe.

Structure:

The lobes of liver are made up of many functional units called lobules. A lobule consists of specialized epithelial cells called hepatic cells or hepatocytes arranged in irregular, branching, interconnected plates around the central vein. Rather than capillaries liver has larger space lined by endothelium called sinusoids through which blood passes. The sinusoids are also partly lined with stellate reticuloendothellial (Kuffer's) cells which phagocytes worm, bacteria and toxic substances. Bile secreted by hepatic cells enters bile capillaries that empty into small bile ducts. These ducts eventually merge to form the larger right and left hepatic duct, which unite and exit the liver as the common hepatic duct. Further this common hepatic duct joins the cystic duct from the gall bladder to form the common hepatic duct. The common hepatic duct and pancreatic duct enter the duodenum in a common duct called the hepatopancreatic ampulla.

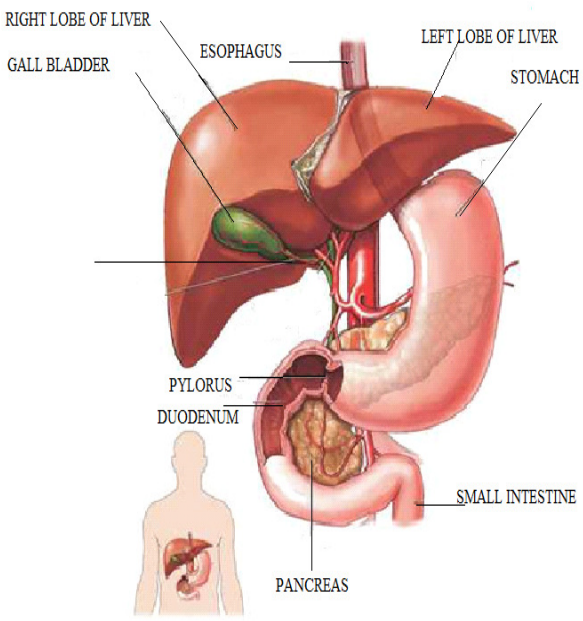


Fig. No.1.1 Liver anatomy[21]

Functions of liver: [20]

1. Secretion and excretion of bile

Bile is partially an excretory product and partially a digestive secretion. Each day the hepatic cell secretes 800-1000 ml of bile, a yellow, brownish or olive green liquid. The principle bile pigment is bilirubin. When worm out red blood cells broken down, iron, globins, and bilirubin (derived from heam) are released.

2. Metabolic functions

A) Carbohydrate Metabolism

After a meal, the liver achieves net glucose consumption (eg, for glycogen synthesis and generation of metabolic intermediates via glycolysis and the tricarboxylic acid cycle). This occurs as a result of a confluence of several effects.

First, the levels of substrates such as glucose increase. Second, the levels of hormones

that affect the amount and activity of metabolic enzymes change. Thus, when blood glucose increases, the ratio of insulin to glucagon in the bloodstream increases. The net effect is increased glucose utilization by the liver. In times of fasting (low blood glucose) or stress (when higher blood glucose is needed), hormone and substrate levels in the bloodstream drive metabolic pathways of the liver responsible for net glucose production (eg, the pathways of glycogenolysis and gluconeogenesis). As a result, blood glucose levels are raised to, or maintained in, the normal range in spite of wide and sudden changes in the rate of glucose input (eg, ingestion and absorption) and output (eg, utilization by tissues) from the bloodstream .

B) Protein Metabolism

Related to its important role in protein metabolism, the liver is a major site for processes of oxidative deamination and transamination. These reactions allow amino groups to be shuffled among molecules in order to generate substrates for both carbohydrate metabolism and amino acid synthesis. Likewise, the urea cycle allows nitrogen to be excreted in the form of urea, which is much less toxic than free amino groups in the form of ammonium ions. Impairment of this function in liver disease is discussed in greater detail later.

C) Lipid Metabolism

The liver is the center of lipid metabolism. It manufactures nearly 80% of the cholesterol synthesized in the body from acetyl-CoA via a pathway that connects metabolism of carbohydrates with that of lipids. Moreover, the liver can synthesize, store, and export triglycerides. The liver is also the site of keto acid production via the pathway of fatty acid oxidation that connects lipid catabolism with activity of the tricarboxylic acid cycle.

In the process of controlling the body's level of cholesterol and triglycerides, the liver assembles, secretes, and takes up various lipoprotein particles. Some of these particles (very low-density lipoproteins [VLDL]) serve to distribute lipid to adipose tissue for storage as fat or to other tissues for immediate use. In the course of these functions, the structure of VLDL particles is modified by loss of lipid and protein components. The resulting low-density lipoprotein (LDL) particles are then returned to the liver by virtue of their affinity for a specific receptor, the LDL receptor, found on the surface of various cells of the body, including hepatocytes. Other lipoprotein particles (high-density lipoproteins [HDL]) are synthesized and secreted from the

liver. They scavenge excess cholesterol and triglycerides from other tissues and from the bloodstream, returning them to the liver where they are excreted. Thus, secretion of HDL and removal of LDL are both mechanisms by which cholesterol in excess of that needed by various tissues is removed from the circulation

3. Haematological functions (Haematopoeisis and coagulation)

1. Production of fibrinogen, prothrombin, heparin, and other clotting factors VII, VIII, IC and C.
2. Destruction of erythrocytes.(at the end of their respective life span)

4. Circulatory function

1. Transfer of blood from portal to systemic circulation
2. Blood storage (regulation of blood volume)

5. Detoxification and protective functions

1. Kupffer cells remove foreign bodies from blood (phagocytosis).
2. Detoxiocation by conjugation, methylation, oxidation and reduction.
3. Removal of ammonia.

6. Drug metabolism

Liver plays a vital role in biotransformation of drugs. It converts drug molecule from non polar to polar. Non polar drugs can be conjugated with more polar compounds, which make it water soluble for the urinary excretion.

Phases of Biotransformation

Biotransformation generally occurs in two phases. Phase I reactions involve oxidation-reductions in which an oxygen-containing functional group is added to the substance to be excreted. While oxidation itself does not necessarily have a major effect on water solubility, it usually introduces into the drug a reactive "handle" that makes possible other reactions that do render the modified substance water soluble.

These phase II reactions usually involve covalent attachment of the drug to a water-soluble carrier molecule such as the sugar glucuronic acid or the peptide glutathione. Unfortunately, by making substances more chemically reactive, phase I oxidation reactions often convert mildly toxic drugs into more toxic reactive intermediates. If conjugation by phase II enzymes is impaired for some other reason, the reactive intermediate can sometimes react with and damage other cellular structures. This feature of drug detoxification has important clinical implications.

Paracetamol induced hepato necrosis:

Paracetamol, a widely used analgesic and antipyretic drug, produces acute liver damage in high doses. Paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. The covalent binding of N-acetyl-P- benzoquinoneimine, an oxidative product of paracetamol to sulphydryl groups of protein, result in lipid peroxidative degradation of glutathione level and thereby, produces cell necrosis in the liver. Dose of Paracetamol: 1 gm/kg P.O[22].

1.2:Liver Diseases:

1. Jaundice [23]

This is the yellow pigmentation of the skin, mucous membrane and deeper tissues due to increased bilirubin level in blood. The normal serum bilirubin level is 0.5 to 1.5 mg%. When this serum bilerubin level exceeds 2 mg %, jaundice occurs.

Types and causes of Jaundice

Jaundice is classified into three type‘s namely haemolytic jaundice, hepatocellular jaundice, and obstructive jaundice.

a) Haemolytic Jaundice

Haemolytic jaundice is also called prehepatic jaundice. During this, the excretory function of liver is normal. But, there is excessive destruction of red blood cells and thus the bilirubin level in blood is increased the liver cells cannot excrete much bilirubin rapidly. So, it accumulates in the blood resulting in jaundice. In this type of jaundice the free bilirubin level increases in blood. Increased in formation of urobilinogenin resulting in the excretion of more

amount of urobilinogenin urine. Any condition that causes haemolytic anemia can lead to haemolytic jaundice.

b) Hepatocellular Jaundice

The jaundice due to the damage of liver cells is called hepatocellular or hepatic jaundice. It is also called hepatic cholestatic jaundice. Here, bilirubin is conjugated. But the conjugated bilirubin cannot be excreted. So, it returns to the blood. The damage of liver cells occurs because of toxic substances (toxic jaundice) or by infection (infective jaundice). Commonly liver is affected by virus resulting inhepatitis.

c) Obstructive Jaundice

This is otherwise called extra hepatic cholestatic jaundice or post hepatic jaundice. It is due to the obstruction of bile flow at any level of biliary system. The bile cannot be poured into small intestine and bile salts and bile pigments enter the circulation. In this, blood contains more conjugated bilirubin.

2. Hepatitis [23]

Hepatitis is a liver disease characterize by swelling and inadequate functioning of liver. Hepatitis may be acute or chronic. In severe conditions, it may lead to liver failure and death.

Causes and Types

Hepatitis is caused by viruses, bacteria poisons, autoimmune disease drug abuse, alcohol, some therapeutic drugs and inheritance from mother during parturition. Viral hepatitis is of five types namely, hepatitis A, B, C, D and E.

Hepatitis A and E are caused mostly by intake of water and food contaminated with hepatitis virus. Generally these two types of hepatitis are not life threatening.

Hepatitis B, C and D are caused by sharing needles with infected person, accidental prick by infected needle, having unprotected sex with infected person, inheritance from mother during parturition and blood transfusion from infected donors.

These three forms of hepatitis are serious diseases when compared to hepatitis A and E. Among these, hepatitis B is more common and considered more serious because it may lead to cirrhosis and cancer of liver.

3. Cirrhosis [24]

The inflammation and damage of parenchyma of liver is known as cirrhosis of liver. This may results in degeneration of hepatic cells and dysfunction of liver.

Cirrhosis is a diffuse, chronic, necrotic (degenerative) liver disorder characterized by progressive hepatocyte injury followed by regeneration and fibrosis leading to disorganization of lobular architecture, pseudo lobule formation and acquired vascular malformation. .

4. Tumours of Liver

a) Benign tumors

i) Benign haemangioma

ii) Cysts

b) Malignant tumors

i) Secondary metastasis is the most common tumors. It may be from breast, lungs and colon.

ii) Primary tumours

5. Hepatocellular Carcinoma

It is the most common primary liver cancers (comprising 90% of all tumors).

Etiology and Pathogenesis

Infection with hepatitis B virus.

Cirrhosis

Environmental toxins e.g. alpha toxins B produced by Aspergillus flavus.

Oral contraceptive.

6. Hepatocellular Failure[23,24]

It may occur due to- Ultra structural lesions of hepatocytes e.g. Raye‘s syndrome. Chronic liver diseases e.g. chronic hepatitis, cirrhosis, Wilson‘s disease,Coma

7. Hepatic Encephalopathy

Also called as hepatic coma, is a feature of chronic liver failure. It is a metabolic disorder of the central nervous system and neuromuscular systemassociated with hepatic failure. It is reversible condition.

8. Portal Hypertension

In this condition, there is increased resistance to portal blood flow. It may occur in the conditions of Portal vein thrombosis, Splenomegaly, Cirrhosis.

For this types of liver diseases the various allopathic drugs are used however in is not completely cured, so there is is a need of drug which is obtained from various natural sources like plants source etc.. for the treatment of liver diseases.

The traditional medicines derived from medicinal plants are used by about 60 % of the world's population. Especially in india, herbal drugs and plants used in the treatment of liver disease.So my studies are carried out by herbal drugs according to this basis of several reasons.

Rahal Widanagamage et al 2008 carried out the study of oral hypoglycemic effect of on leaves of *Canthium coromandelicum* (Burm.f)Alston. Using various organic solvent (pet ether, ethyl acetate ,methanol) extracts reported that it shows oral hypoglycemic activity.[25]

Santhosh kumar et al 2013 reported that the anti microbial and anti HIV activity of in *Canthium coromandelicum* (Burm.f) Alston leaves in rats. And reported that the methanolic extract have anti microbial activity againt gram positive, gram negative and fungus micro organisms by zone of inhibition method.[26]

Gupta M et al, Antioxidant and Hepatoprotective effects of *Bauhinia racemosa* against Paracetamol and CCl4- induced liver damage in rats[27].

Pramyothin P et al, have investigated the hepatoprotective effect and possible mechanism of aqueous extract of *Phyllanthus amarus Schem.et Thonn*.(PA)[28]

Divya balne et al, 2013 carried out that hepatoprotective effect of whole plant extract fractions of *Marsilea minuta linn* by using paracetamol and ccl_4 induced hepatotoxicity in rats and reported that it has significant hepatoprotective activity.[29]

Sabitha et al ,2011 have investigated the hepatoprotective and hypoglycemic effect of methanol and aqueous extract of the whole plant *Sphaeranthus zeylanicus* and reported that, both the methanol and aqueous extracts showed significant anti-diabetic activity and hepatoprotective activity.[30]

Smita shenoy et al, 2012 carried out that hepatoprotective effect of *Plectranthus amboinicus* against paracetamol induced hepatoprotective activity in rats.[31]

B.Shyam kumar et al, 2010 was investigated hepatoprotective activity of leaves of *Coccinia indica* in albino rats and reported that it has hepatoprotective activity.[32]

Sutar Niranjan et al, 2012 was carried out the diuretic activity of various extract of *Achyranthes aspera* leaves extract and reported that the aqueous and methanol extract have diuretic activity as similar that of standard frusemide drug.[33]

Umang patel et al, 2009 have investigated the diuretic effect of methanol and aqueous extract of the dried seeds of *Lepidium Sativum* in wistar rats and reported that, both the methanol and aqueous extracts showed significant diuretic activity [34]

Eswaraiah et al, 2013 was investigated that the diuretic activity of ethanolic extract of leaves of *Rhynchosia beddomei* and reported that it possess significant diuretic activity.[35]

AIM OF WORK

Canthium coromandelicum is used traditionally for the treatment of diseases like HIV, diseases produced by micro organism , Diabetics, etc and is known as reputed drug of ayurveda.

Our aim is to prepare the methanolic and aqueous extract of the leaves *Canthium coromandelicum* and to perform biological screening to elucidate the therapeutic potential of the plant considering their traditional usage, the following objectives are

1. Qualitative phyto chemical evaluations of *Canthium coromandelicum*

2. Hepatoprotective model.

3. Diuretic model.

Plan of Work

The present study planned in the following order to assess the Hepatoprotective and diuretic activities of *Canthium coromandelicum* .

1. Collection and authentication of the plant.
2. Extraction of leaves of *Canthium coromandelicum* .
3. Preliminary phytochemical evaluation
4. Acute toxicity studies.
5. The estimation of the following parameter for the evaluation of Hepatoprotective activity.
    - SGOT
    - SGPT
    - Total bilerubin
    - Wet liver weight
    - Histopathological studies

6.The estimation of the following parameter for the evaluation of Diuretic activity.

- Urine volume
- Diuretic index
- pH
- Electrolyte levels such as sodium and potassium.

PLANT PROFILE [36]

Name:	*Canthium coromandelicum*
Kingdom	Plantae
Phylum	Magnoliatae
Class	Magnoliatae
Order	Rubiales
Family	Rubiaceae
Sub Family	Lxoroideae
Genus	*canthium*

COMMON NAME: [37]

Tamil : Nallakarai, Sengakarai.

Malayalam : Kantankara, Serukara.

Telugu : Balusu, Sinnabalusu.

English : Carray cheddie, Wild Jessamine.

Kannada : Karemullu, Ollepode.

Botanical name: *Canthium coromandelicum*

PLANT DESCRIPTION:

Canthium coromandelicum is a Armed shrubs; branchlets obtusely 4-angled; bark grey; spines 1-3.2 cm long, supra-axillary. Leaves 1.8-4.2 x 1.3-3 cm, ovate, elliptic-ovate to obovate, base rounded to attenuate, apex subacute; petiole c. 5 mm long; stipules subulate. Flowers greenish, 4-merous, in axillary, sessile cymes below spines. Calyx cupular, 4-toothed. Corolla 4-5 mm across, campanulate to globose, mouth villous; lobes 4, ovate, acute, spreading or reflexed. Stamens 4, subsessile, exserted. Stigma capitate, slightly 2-lobed. Fruit 1-1.4 cm across, subglobose, yellow.

Flower

In axillary, decussate, lax cymes;greenish. Flowering from May-August.

Fruit

A globose drupe; orange when ripe; pyrenes furrowed. Fruiting July onwards.

Field tips

Spines supra-axillary. Fruit flattened with a longitudinal groove.

Leaf Arrangement

Opposite-decussate

Leaf Type

Simple

Leaf Shape

Elliptic-ovate to obovate

Leaf Apex

Subacute

Leaf Base

Attenuate

Leaf Margin

Entire

MATERIALS AND METHODS

LIST OF CHEMICALS USED

S.NO	CHEMICALS	SOURCE
1	Methanol	CENTRAL DRUG HOUSE (P) LTD NEW DELHI
2	Petroleum ether	CENTRAL DRUG HOUSE (P) LTD NEW DELHI
3	Ethyl acetate	CENTRAL DRUG HOUSE (P) LTD NEW DELHI
4	chloroform	CENTRAL DRUG HOUSE (P) LTD NEW DELHI
6	SGOT KIT	GIRI DIAGNOSTIC KIT PVT LTD , UTTARPRADESH
7	SGPT KIT	GIRI DIAGNOSTIC KIT PVT LTD , UTTARPRADESH

S.NO	DRUGS	COMPANY
1	Paracetamol	XYKAA 650, Troikaa Pharmaceuticals ltd. Ahemadabad
2	Silymarin	SILYBON, MICROLABS
3	Frusemide	FRUSEMENE GSK

LIST OF DRUGS USED

All the substance are prepared immediately before use and the reagents were used as analytical grade.

Plant Materials:

The Leaves of the plant *Canthium coromandelicum* were collected at srivilliputtur during the month of july 2013. It was then authenticated by Dr.V Ganesan M.Sc Ph.D, Department of Botany, The Ayya nadar janaki ammal College, Sivakasi.

Extraction[38-39]

Extraction involves the separation of bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedure**.**

The coarse powder was extracted by soxhlet apparatus with Petroleum ether, Chloroform, Methanol and distilled water for 48 hours. The extracts were collected by filtration, the marc was separated and the extraction was repeated with fresh solvents for two times. The extracts were combined and concentrated at 55°C on water bath, till it acquires a maximum concentration.

Then a small fraction of all the extracts were subjected to various chemical tests for the identification of various plant constituents as in the procedure given below and the findings are reported in table.

Petroleum ether extract of leaves of *Canthium coromandelicum:*

The dried coarse powder of leaves of *Canthium coromandelicum* was extracted with 1 litre of petroleum ether (60-80 ^{0} C) by continuous percolation method using soxhlet apparatus, After 24 hours the extraction was completed then petroleum ether was taken and the solvent was redistilled , A dark green colour residue was obtained.

Chloroform Extract of leaves of *Canthium coromandelicum:*

The marc left after extraction, was dried and subsequently extracted with 1 litre of choloroform by continuous percolation method. After 24 hours the extraction was completed, it was filtered and the solvent was removed by distillation under reduced pressure. The green coloured residue was stored in a desiccator and the marc was dried for further extraction.

Methanol Extract of leaves of *Canthium coromandelicum:*

The marc left after extraction, was dried and subsequently extracted with 1 litre of methanol by continuous percolation method. After 24 hours the extraction was completed, it was filtered and the solvent was removed by distillation under reduced pressure. The green coloured residue was stored in a desiccator and the marc was dried for further extraction.

Aqueous Extract of leaves of *Canthium coromandelicum:*

The marc left after Chloroform form extraction, was dried and subsequently extracted with 1 litre of Aqueous by continuous hot percolation method. After the completion of extraction it was filtered and solvent was redistilled, and then greenish yellow coloured residue was stored in a desiccators.

Extraction of the leaves of *Canthium coromandelicum*

S.NO	Extracts	Colour and Consistency	Percentage Yield of Extracts of *Canthium coromandelicum* w/w
1.	Petroleum ether	Dark Green $ Viscous mass	0.68
2.	Methanol	Green with sticky mass	1.85
3.	Aqueous	Greenish Yellow Colour	0.92
4	Chloroform	Dark pink colour	0.30

PRELIMINARY PHYTOCHEMICAL STUDIES:[40]

The various extracts of *Canthium coromandelicum* obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, triterpenoids, phenols, proteins, tannins etc.

PROCEDURE:

1. TESTS FOR ALKALOIDS:

The small portion of the Petroleum ether, Chloroform, Methanol and Aqueous extracts were dissolved in suitable solvent and each extract was stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested for alkaloids by using the following reagents.

a. **Mayer's reagent** (potassium mercuric iodide)

b. **Dragendroff's reagent** (potassium bismuth iodide)

c. **Wagner's reagent** (iodine + potassium iodide)

d. **Hager's reagent** (saturated picric acid)

2. TESTS FOR CARBOHYDRATES:

A small portion of the Petroleum ether, Chloroform, Methanol and Aqueous extracts were dissolved separately in 5ml of water and filtered. The filtrate was subjected to the following tests.

a. Molisch Test :

To a small portions of the Petroleum ether, Chloroform, Methanol and Aqueous extracts alpha-naphthol in alcohol followed by conc. H_2SO_4 was added through the sides of the test tube.

b. Fehling's solution :

To a solution of the Petroleum ether, Chloroform, Methanol and Aqueous substances, a mixture of equal parts of Fehling's solution A and B was added and the test tube was heated on a water bath.

c. Barfoed's test:

To a small portion of the Petroleum ether, Chloroform, Methanol and Aqueous substances, Barfoed's solution was added and it was boiled.

d. Benedict's test:

To a small portion of the Petroleum ether, Chloroform, Methanol and Aqueous substances, Benedict's solution was added and mixed well and it was boiled. Then it was allowed to cool.

3. TESTS FOR GUMS AND MUCILAGES:

a. To a small amount of Petroleum ether, Chloroform, Methanol and Aqueous extracts, 25ml of absolute alcohol was added and then it was filtered. The precipitate was examined for its swelling properties.

b. To the Petroleum ether, Chloroform, Methanol and Aqueous extracts, ruthenium red solution was added.

4. TESTS FOR SAPONINS:

a) Foam Test :

1 ml of the Petroleum ether, Chloroform, Methanol and Aqueous extract solutions was taken in a measuring cylinder. To this, 20 ml of distilled water was added and shaked well.

b) Haemolysis Test:

The Petroleum ether, Chloroform, Methanol and Aqueous extracts of the plant was spread over a glass slide to form a thin film layer on which a drop of human blood was placed and spread over the extract layer. After 30 minutes, the slide was examined under microscope for change in the structure and shape of red blood cells. Control was always maintained to see the change in red blood cells structure for haemolysis.

5. TESTS FOR FIXED OILS AND FATS:

a. Spot Test : To the Petroleum ether, Chloroform, Methanol and Aqueous Extracts of plant was taken and they were pressed between filter paper and the paper was noted.

b. Few drops of 0.5N alcoholic potassium hydroxide was added to Petroleum ether, Chloroform, Methanol and Aqueous extracts with few drops of phenolphathalein. The mixture was heated on a water bath for 1-2 hours.

6. TESTS FOR TRITERPENOIDS:

Salkowski Test : The Petroleum ether, Chloroform, Methanol and Aqueous extracts was taken and it was added with chloroform and sulphuric acid and the fluorescence of the solution was noted.

7. TEST FOR TRITERPENES:

The Petroleum ether, Chloroform, Methanol and Aqueous extracts was taken and it was added with 2ml of chloroform, 10ml acetic anhydride and 2 drops of conc. sulphuric acid.

8. TESTS FOR PROTEINS AND AMINO ACIDS:

a. Millon's Test:

The Petroleum ether, Chloroform, Methanol and Aqueous Extracts of leaves was added with Millon's reagent and it was boiled.

b. Ninhydrin test:

The Petroleum ether, Chloroform, Methanol and Aqueous Extracts of leaves was added with Ninhydrin solution and it was boiled. Then it was allowed to cool.

c. Biuret Test:

The Petroleum ether, Chloroform, Methanol and Aqueous Extract was added with Biuret reagent and colour were observed.

d. Xanthoprotein Test:

The Petroleum ether, Chloroform, Methanol and Aqueous extracts, concentrated nitric acid was added and the change were observed.

9. TEST FOR PHYTOSTEROLS:

The Petroleum ether, Chloroform, Methanol and Aqueous extracts was refluxed separately with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted with distilled water and extracted with ether. The etherial extract was evaporated and the residue was subjected to Libermann- Burchard test.

LIBERMANN-BURCHARD TEST:

The Petroleum ether, Chloroform, Methanol and Aqueous Extracts was shaken with few drops of dry acetic acid. To this, 3ml of acetic anhydride was added followed by 3 drops of conc. sulphuric acid.

10. TEST FOR PHENOLIC COMPOUNDS AND TANNINS:

Small quantities of Petroleum ether, Chloroform, Methanol and Aqueous extracts was taken separately in water and the presence of phenolic compounds and tannins were tested by adding with dilute.$Fecl_3$ solution(5%)/ 10% Lead acetate solutions.

11. TEST FOR GLYCOSIDES:

a. Baljet' s Test: The Petroleum ether, Chloroform, Methanol and Aqueous extracts, sodium picrate solution was added.

b. Legal's Test: The Petroleum ether, Chloroform, Methanoland Aqueous extracts, few ml of pyridine, 2 drops of nitroprusside and a drop of 20% Na0H solution was added.

c. Borntrager's Test : The Petroleum ether, Chloroform, Methanol and Aqueous extracts was mixed with dilute.H_2So_4 filtered. The filtrate was shaken with chloroform and the chloroform layer was separated. To this dilute ammonia was added .

12. TEST FOR FLAVONES :

Flavonone:

a. The Petroleum ether, Chloroform, Methanol and Aqueous Extracts was treated with NaOH, the formation of yellow to orange colour, indicates the presence of flavonone.
b. The Petroleum ether, Chloroform, Methanol and Aqueous Extracts was treated with concentrated H_2So_4 acid the formation of orange to crimson red colour, indicates the presence of flavonone.

Flavones:

a. The Petroleum ether, Chloroform, Methanol and Aqueous Extracts was treated with NaOH, the formation of yellow colour indicates the presence of flavone.
b. The Petroleum ether, Chloroform, Methanol and Aqueous Extracts was treated with concentrated H_2So_4 acid the formation of yellow to orange colour, indicates the presence of flavone.

Acute toxicity studies:

Determination of acute toxicity (LD50): [41-44]

14 days single dose oral acute toxicity and gross behavioural study 103-106

Number of animals required: 6 mice (male)

Number of groups: 2 groups (3 animals each group)

Dose levels: 4000 mg/kg body weight of the animals.

Study duration: 14 days

Preparation of dose:

Methanolic and Aqueous extract of *Canthium coromandelicum* leaves was suspended in 3% CMC, to prepare a dose of 4000 mg/kg body weight of animal, and administered 1ml/100gm body weight of the animal.

Procedure:

The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Two set of healthy female animal (each set of 3 mice) were used for the experiment. First set animals were divided and fasted for 18 hours deprived from food, water withdrawn before 4 hours of the dosing, body weights were noted before and after dosing with Methanolic and

Aqueous extract of *Canthium coromandelicum* (4000 mg/kg) orally. Individually animals were observed for 4 hours to see any clinical symptoms, any change in behaviour or mortality. 6 hours post dosing again body weights recorded. Form the next day onwards, each day for 1 hour the behavioural change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors.

FLOW CHART

Annexure – 2c – OECD Guidelines: Test Procedure Starting Dose of 300 mg/kg B.W.

START

⇩

300 mg/ kg / 3 animals

⇩

0 - 1 (None of animals died)

⇩

2000 mg/ kg

⇩

2 – 3 (None of animals died)

⇩

3000 mg/kg (none of animals died)

⇩

4000 mg/ kg / 3 animals

⇩

None of animals died

⇩

LD50 cut off 4000 mg/ kg B.W.

Separation and Isolation of plant Constituents by Chromatographic Methods[45]

The various methods of separating and isolating the plant constituents, the chromatographic procedure originated by Tswettis one of most useful technique for general application. All finely divided solids have the powder to adsorb other substance are capable of being adsorbed, some much more readily than others, this phenomenon of selective adsorption is the fundamental principle of chromatography. In the present study, thin layer chromatography methods were used.

Thin Layer Chromatography:

Thin Layer Chromatography is so widely used that it has become an essential technique for analyst and research workers. TLC is the almost universal analytical technique in chemical analysis for organic and inorganic matter.

TLC is a simple and rapid method carried out using thin layer of adsorbents on plates. TLC not only combines the advantage of paper and column chromatography but in certain aspects it is found to be superior to either method.

TLC is an important tool in the separation, identification and estimation of different classes of natural products. When a mixture containing different components is made to ascend in a TLC plate with the help of a solvent which acts as mobile phase, there will be a preferential adsorption of different components at different places on the plate. The result is the separation of components.

Preparation of TLC Plate:

80 gram of silica gel G was weighed and shaken to a homogenous suspension with 85 ml of distilled water for 90 seconds. This suspension was poured in TLC applicator which was adjusted to 0.25 mm thickness 20 carriers the transparence of layer disappeared. The plates were dried in hot air oven at 110^0 C for 30 minutes (activation). The plates were then stored in a dry atmosphere and used whenever required.

Application of extracts for separation:

The various diluted extracts spotted on a TLC plate 2 cm above its bottom using capillary tube. Most solution for application were between 0.1 – 1 % strength. The starting point was equally sized as far possible and spots had diameter ranging from 2 – 5 mm.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

The various extracts of fruits powder of *Canthium coromandelicum* were subjected to Thin Layer Chromatography using different mobiles phases that are suitable for detecting various phytoconstituents like alkaloids, glycosides, flavonoids, tannins and phenols of the three extracts ,

Chromatogram for the petroleum ether,chloroform methanol, Aqueous was carried out using the procedure recommended by Indian pharmacopeia.

HEPATOPROTECTIVE ACTIVITY[46-49]

Male Albino rats weighing between 160-180 gm used in the experiment were kept in animal house under standard environmental conditions and had free access to feed and water *ad libitum*. The animals were fasted for 16 hours before experiment but allowed free access to water.

The rats were divided into six groups each containing four rats.

Group I : Control group of animals received Normal saline (10 ml/kg)

Group II : treated with silymarin (200 mg/kg)

Group III : treated with methanol Extract of leaves of *Canthium coromandelicum* (200 mg/kg)

Group IV : treated with methanol Extract of leaves of *Canthium coromandelicum* (400 mg/kg)

Group V : treated with aqueous Extract of leaves of *Canthium coromandelicum* (200 mg/kg)

Group VI : treated with Aqueous Extract of leaves of *Canthium coromandelicum* (400 mg/kg)

The treatment was continued for seven days. On 8th day a single dose of paracetamol (1000 mg/kg) suspension was given to Groups II –VI. After 48 hrs of paracetamol administration, blood was collected from all the groups of rats by direct cardiac puncture. The blood samples were allowed to clot for 45 mins at room temperature. Serum was then separated by centrifugation at 2500 rpm at 37°c for 10 mins and analyzed for the biochemical parameters such as SGOT and SGPT.

$$\text{Enzyme level} = \frac{\text{absorbance of test}}{\text{absorbance of normal}} \times 1746(\text{factor})$$

$$\text{Percentage activity} = \frac{\text{standard}}{\text{test}} \text{x} 100$$

DIURETIC ACTIVITY

METABOLIC CAGE METHOD :

Male albino rat weighing about 180-200 gm will be divided into six groups of three animals each. The dosage of drugs was administered to the different groups.

Group I : Control group of animals received Normal saline (10 ml/kg)

Group II : treated with Frusemide (20 mg/kg)

Group III : treated with methanol extract of *Canthium coromandelicum* (200 mg/kg)

Group IV : treated with methanol extract of *Canthium coromandelicum* (400 mg/kg)

Group V : treated with aqueous extract of *Canthium coromandelicum* (200 mg/kg)

Group VI : treated with aqueous extract of *Canthium coromandelicum* (400 mg/kg)

Before conducting the experiments the animals were fasted overnight (18 hrs) prior to the test but free acess to water only and immediately after administration,the rats were paired and placed in the metabolic cages. Urine was collected in a graduated cylinder and it\s volume was recorded at 2 hrs interval for 8 hrs. The cumulative urine excretion was calculated in relation to body weight and electrolye concentration, pH was calculated.

HISTOPATHOLOGICAL STUDIES

Histopathology: [34,35,36,37,38]

A portion of liver tissue in each group was fixed in 10 % formalin and proceded for histopathology.

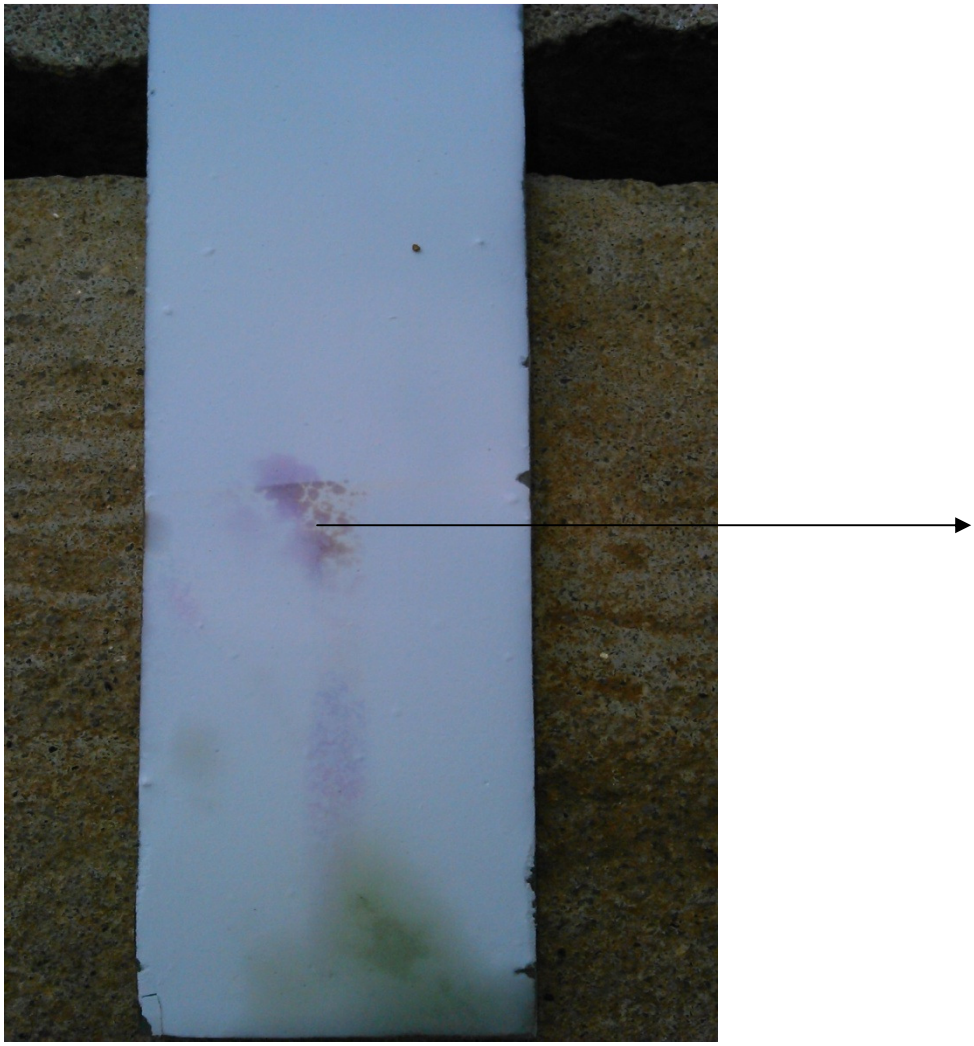
RESULTS

PHYTOCHEMICAL SCREENING ON VARIOUS EXTRACTS OF *CANTHIUM COROMANDELICUM*

Sl. No	Test	Extracts			
		PECC	CECC	MECC	AECC
1. 1	CARBOHYDRATES	–	–	+	–
2.	ALKALOIDS	+	–	+	–
3.	GUMS AND MUCILAGES	–	+	+	+
4.	SAPONINS	–	+	–	+
5.	FIXED OILS AND FATS	–	–	–	–
6.	TRITERPENOIDS	–	–	+	–
7.	TRITERPENES	–	–	+	–
8.	PROTEINS AND AMINO ACIDS	+	+	+	+
9.	PHYTOSTEROLS	–	–	+	+
10.	PHENOLIC COMPOUNDS AND TANNINS	–	–	+	–
11.	GLYCOSIDES	–	–	+	+
12.	FLAVONES	–	–	+	+

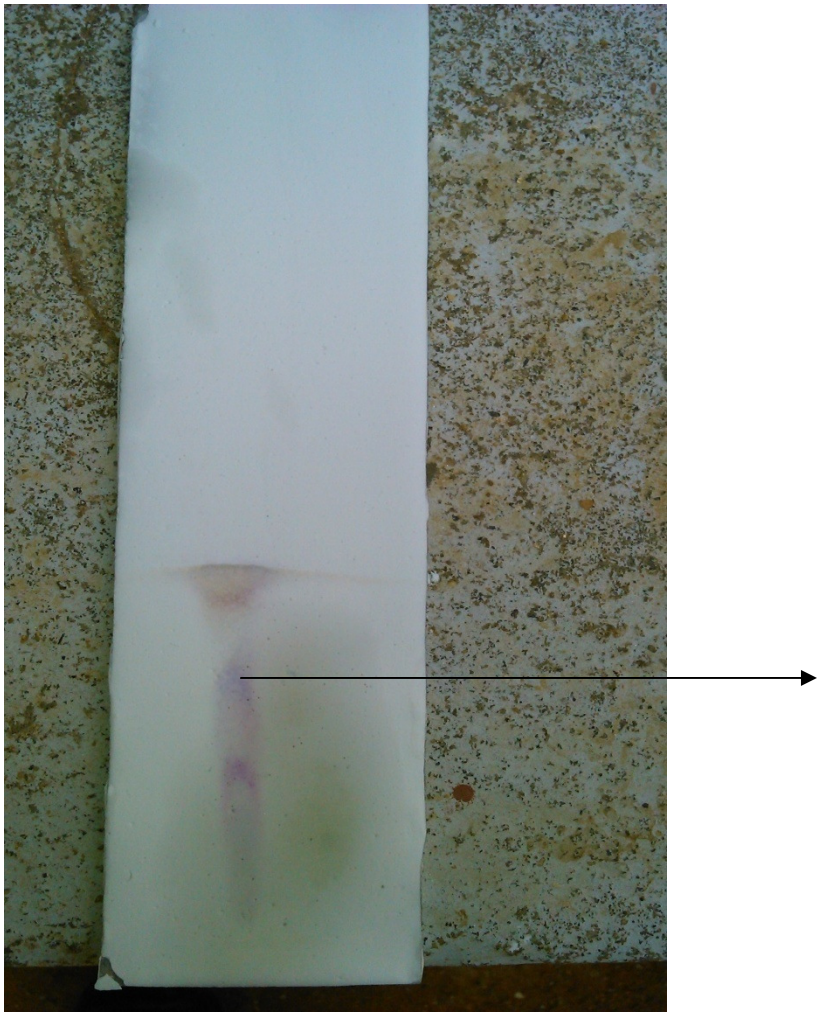
(+) = indicates the presence of constituents, (-) = indicates the absence of constituents

TLC of Various Extracts of *Canthium coromandelicum* leaves.



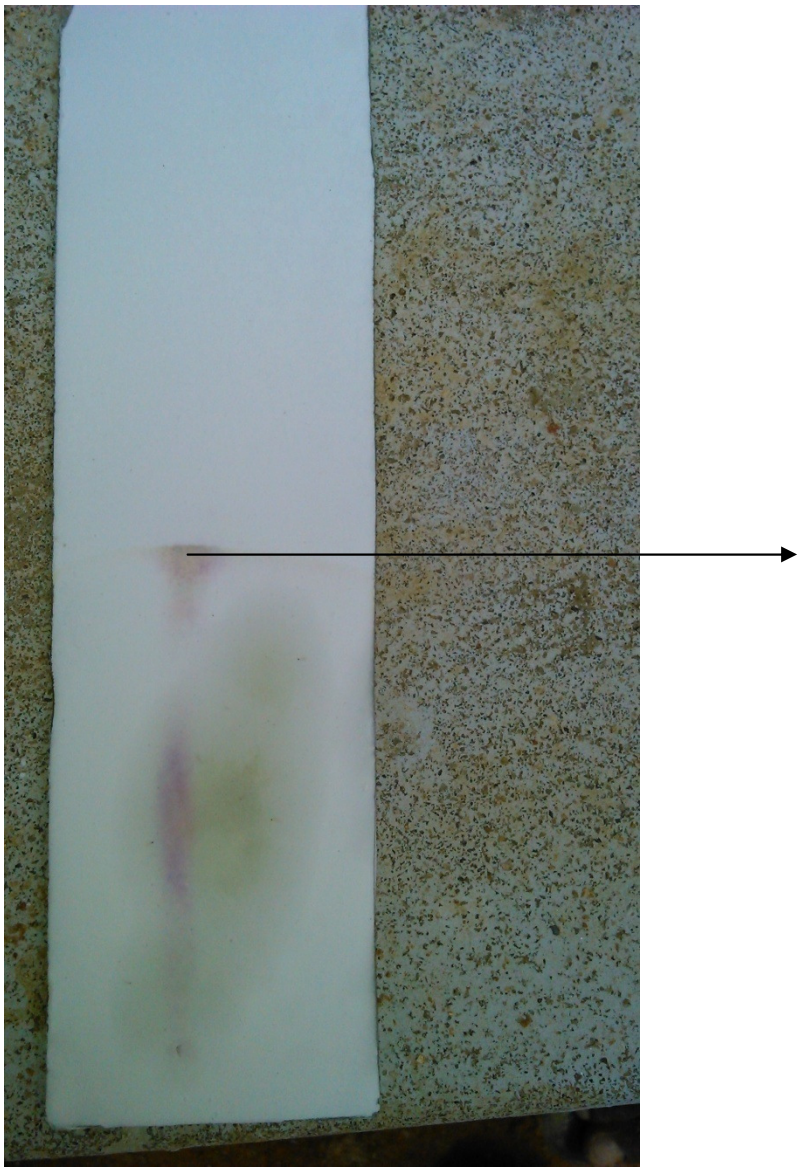
R_f value = Distance travelled by the solute / Distance travelled by the solvent

= 7.8/10 = 0.78



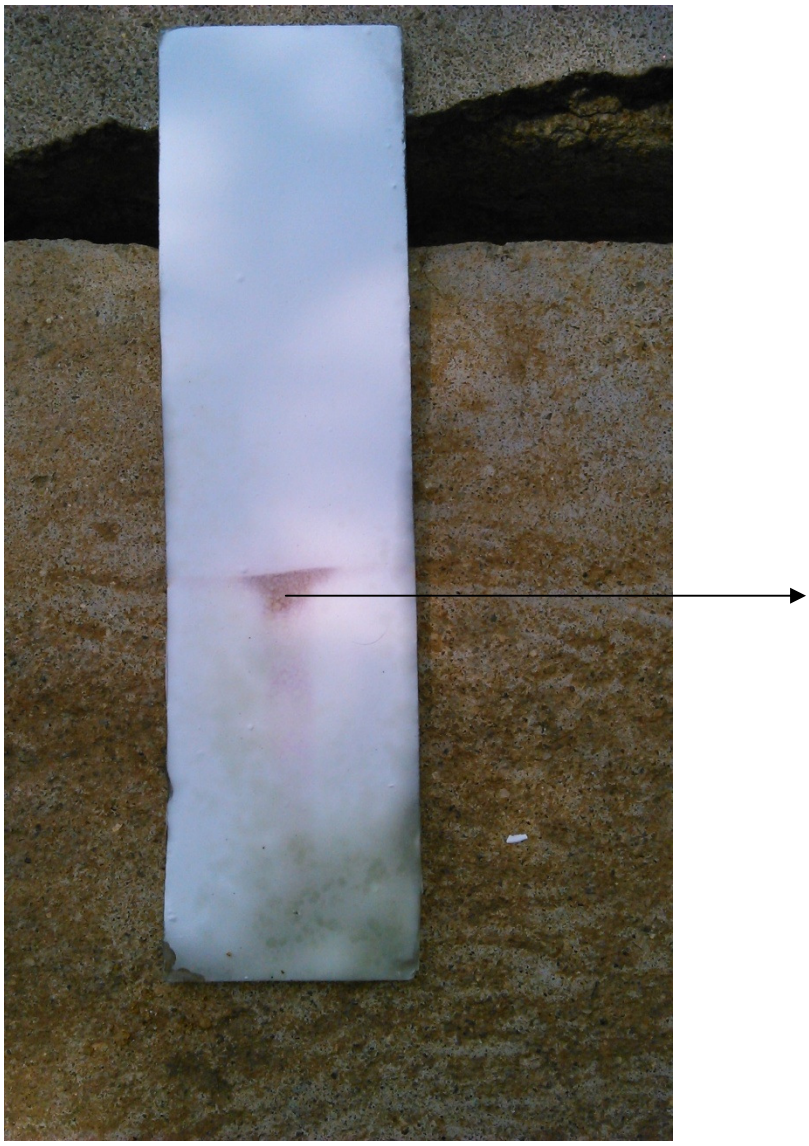
R_f value = Distance travelled by the solute / Distance travelled by the solvent

= 5.6/10 = 0.56



$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

$$= 8.3/10 = 0.83$$



$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

$$= 9.5/10 = 0.95$$

R_f value = $\frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$

= 9.2/10

= 0.92

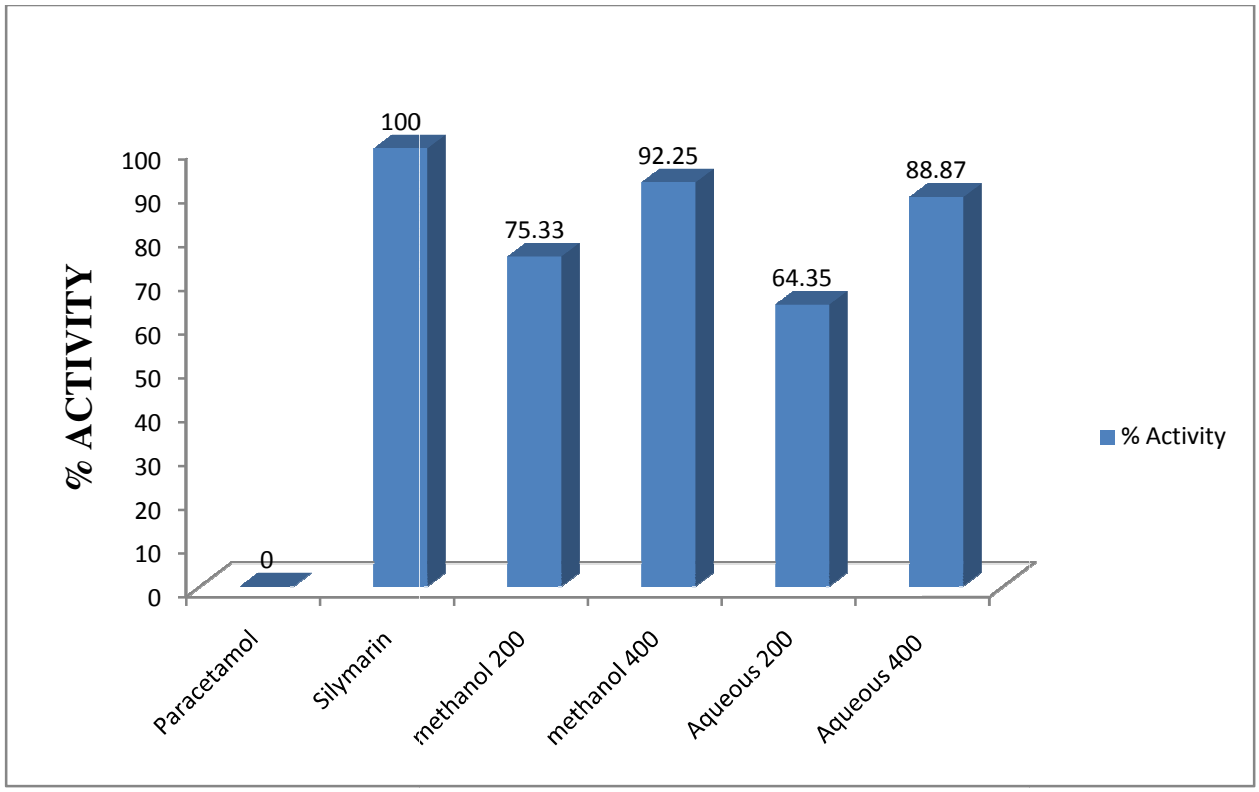
R_f values of various extract of *Canthium coromandelicum* leaves

S.no	Extracts	R_f value
1	MECC	0.78
2	AECC	0.56
3	PECC	0.83
4	CECC	0.92

Table : Effect of the leaves extracts of *Canthium coromandelicum* on paracetamol - induced hepatotoxicity

Treatment	Dose (mg/kg)	SGOT level mean ± SEM	% Activity	SGPT level mean ± SEM	% Activity	Total Bilerubin mean ± SEM
Paracetamol	1000	2097.90 ± 2.46	-	2263.36 ± 1.46	-	2.61 ± 0.16
Standard (Silymarin)	200	1759.53 ± 2.33	100	1870.98 ± 2.65	100	1.47 ± 0.01
MECC	200	2335.64 ± 1.73	75.33	2378.34 ± 2.22	78.66	1.82 ± 0.03
MECC	400	1907.26 ± 1.99	92.25	2090.66 ± 2.32	89.49	1.53 ± 0.01
AECC	200	2734.25 ± 1.35	64.35	2452.67 ± 2.17	76.28	1.96 ± 0.08
AECC	400	1979.96 ± 2.34	88.87	2054.64 ± 1.83	91.06	1.64 ± 0.04

SGOT


% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
0
100
75.33
92.25
64.35
88.87
Paracetamol
Silymarin
methanol 200
methanol 400
Aqueous 200
Aqueous 400
% Activity

SGPT

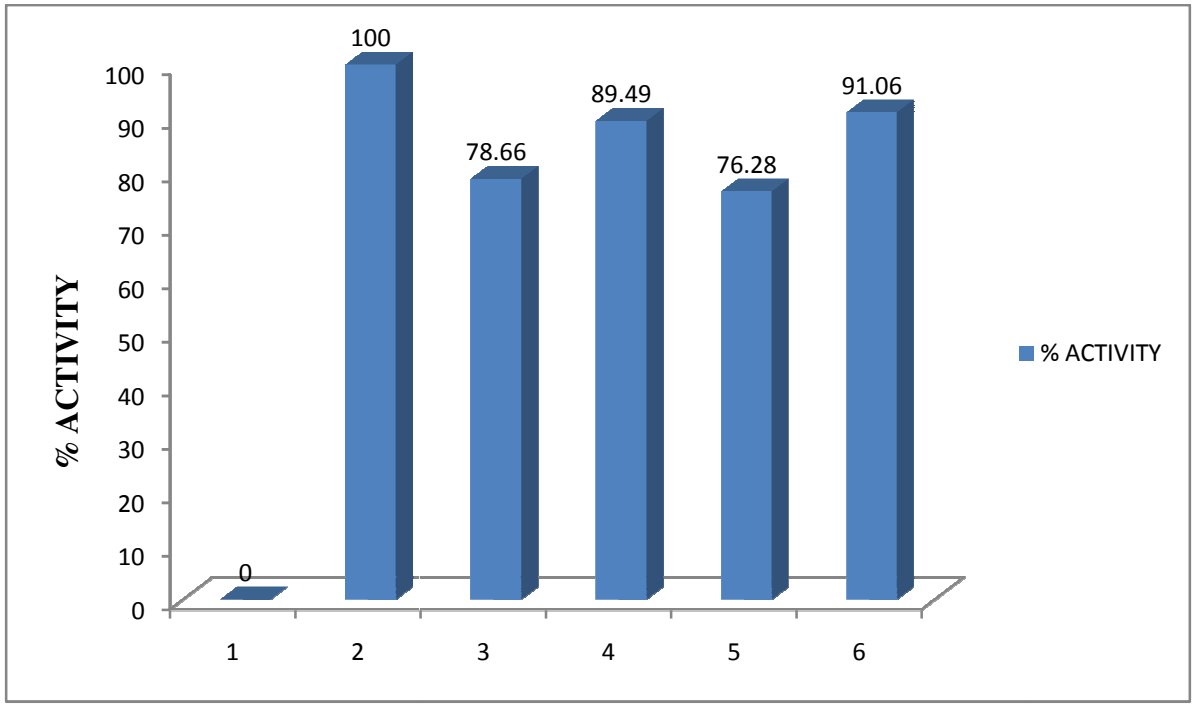
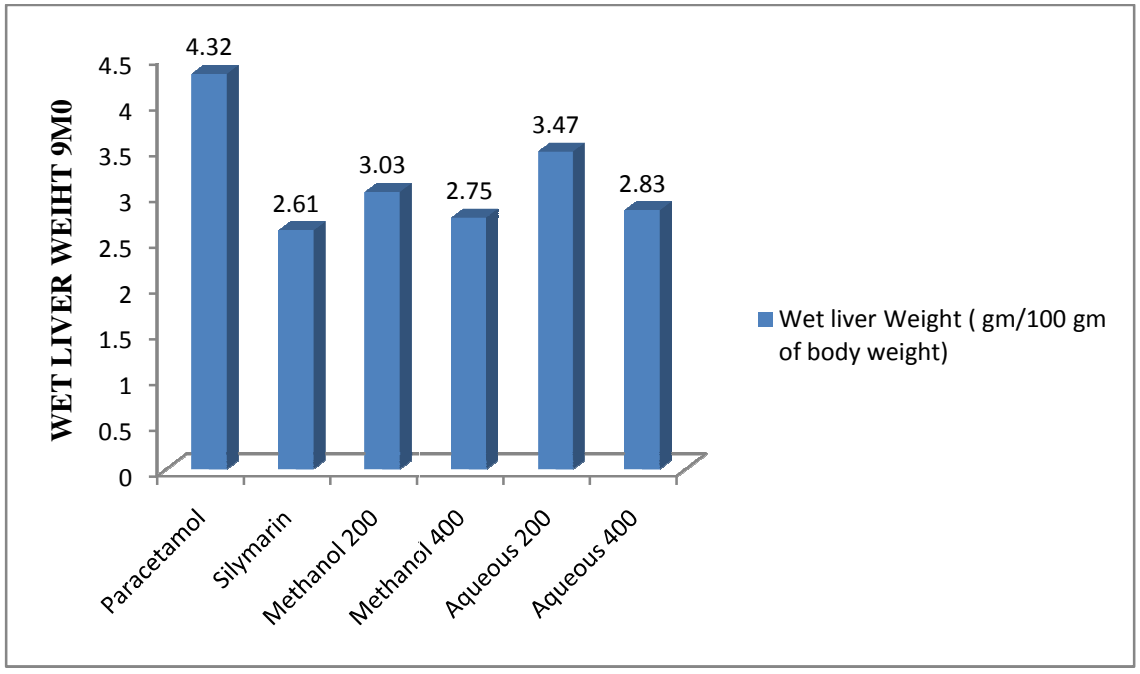

100
89.49
91.06
78.66
76.28
0
% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
% ACTIVITY

Table : Effect Methanolic and aqueous extracts of *Canthium coromandelicum* on paracetamol - induced hepatotoxicity

Treatment	**Dose (mg/kg)**	**Wet liver weight (gm/100gm of Animal) mean ± SEM**
Paracetamol	1000	4.32 ± 0.085
Standard (Silymarin)	200	2.61 ± 0.110
MECC	200	3.03 ± 0.13
MECC	400	2.75 ± 0.15
AECC	200	3.47 ± 0.17
AECC	400	2.83 ± 0.11


WET LIVER WEIHT 9M0
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
4.32
2.61
3.03
2.75
3.47
2.83
Paracetamol
Silymarin
Methanol 200
Methanol 400
Aqueous 200
Aqueous 400
Wet liver Weight (gm/100 gm of body weight)

Histopathological studies of the liver in paracetamol induced hepatotoxicity

The histopathological evaluation of paracetamol toxicity in all the groups was examined and shown in figure. The description is as follows, Section of rat liver treated with vehicle control group shows liver parenchyma with intact architecture which is the normal appearance. Section of liver in toxicant control group shows partially effaced architecture. Some of the hepatocytes show apoptotic changes, perivenular mononuclear inflammatory infiltration, scattered inflammatory infiltration within the parenchyma which is due to toxicity. Section of liver in silymarin treated group shows liver parenchyma with intact architecture. Some of the central veins show congestion with diffuse congestion of sinusoids.

Section of liver in test drug methanol and aqueous treated groups shows intact architecture, few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells which is similar to silymarin treated group.

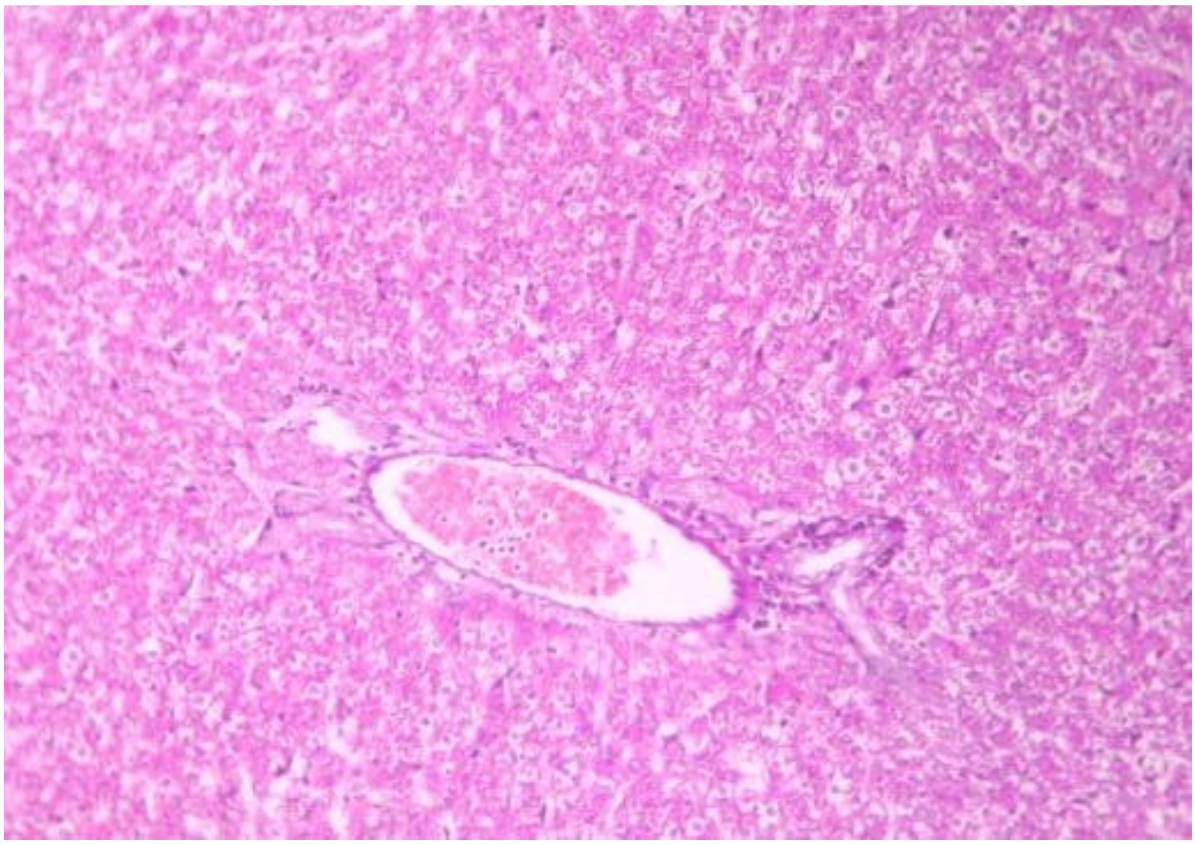


Fig : 1 Normal Contol group, showing normal hepatocytes.

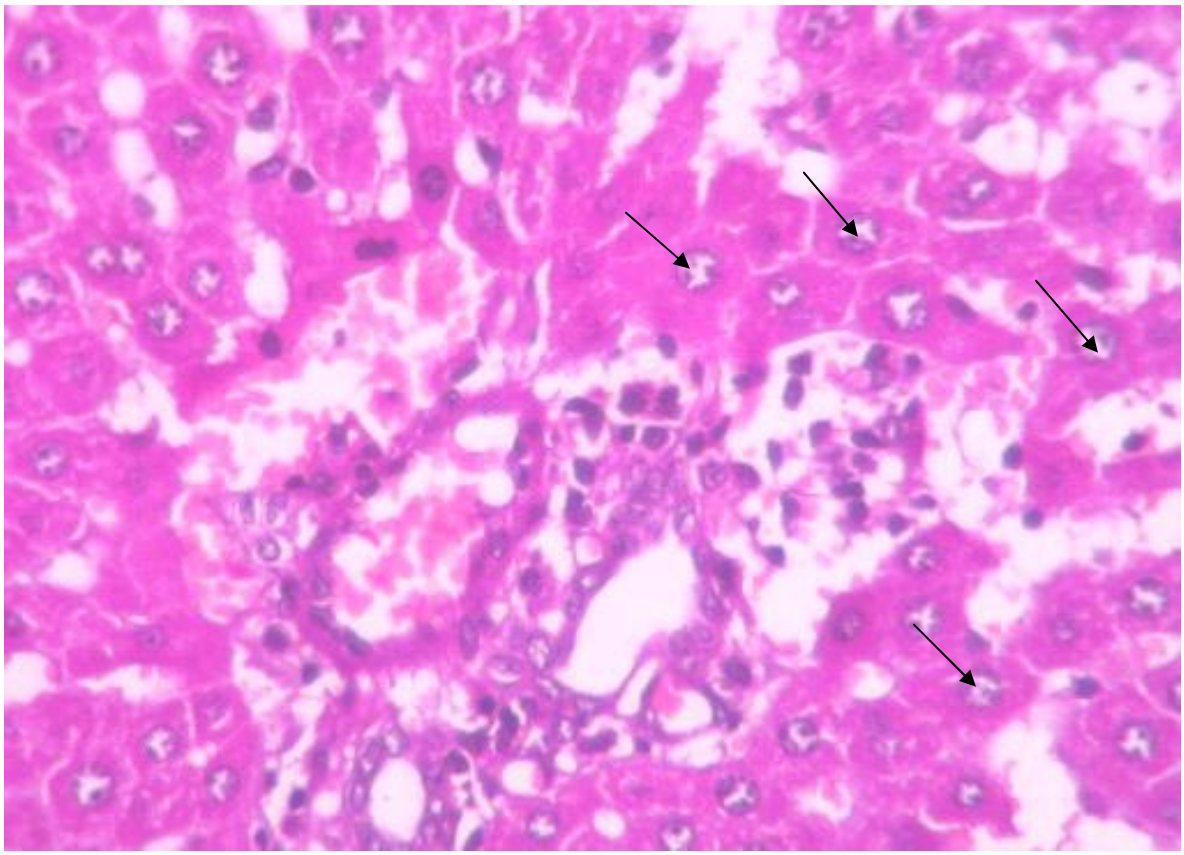


Fig :2 Paracetamol treated animal group shows that hepatic cells damage and congestion of the liver.

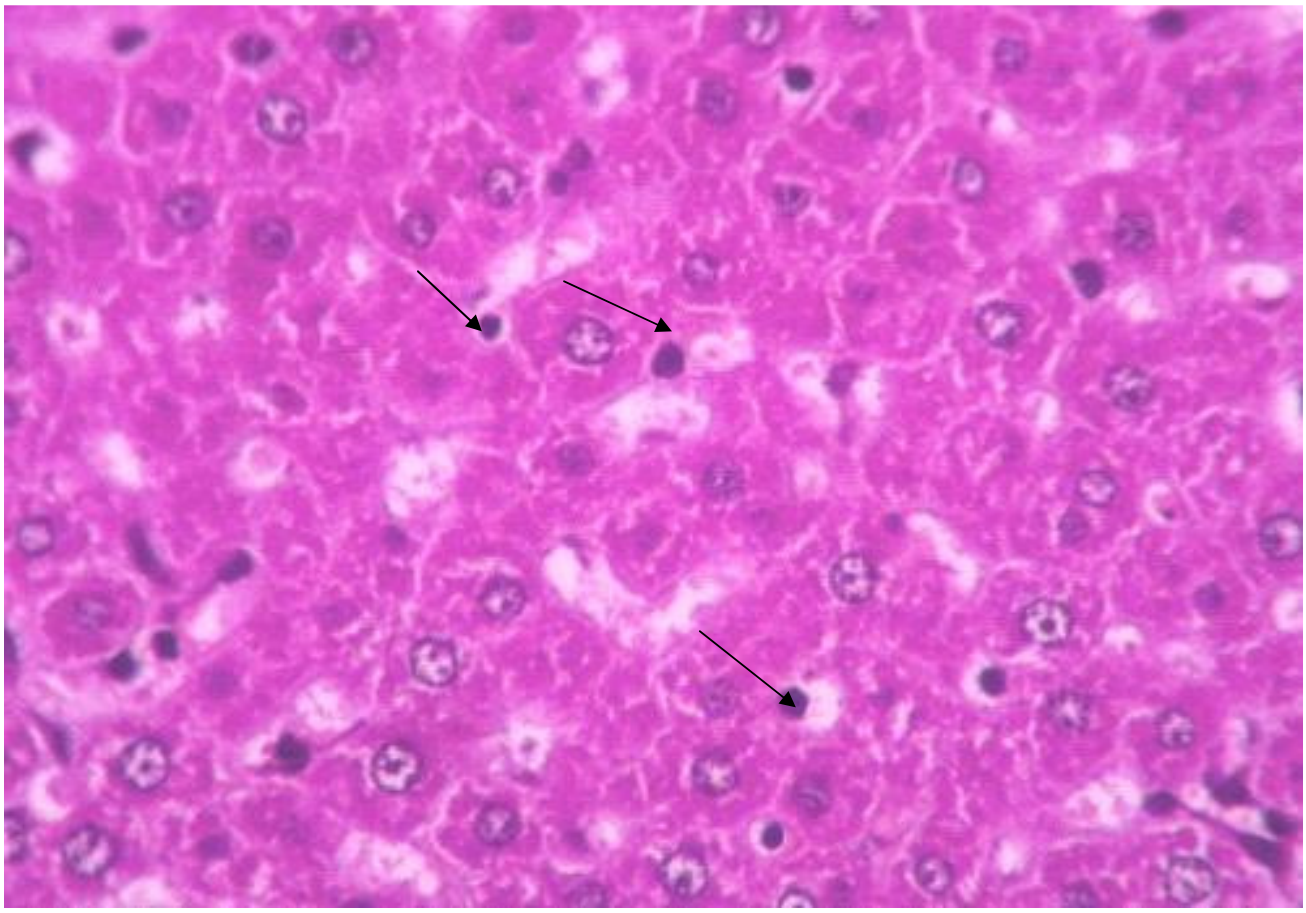


Fig 3 : Hepatocytes in group treated with Standard (Silymarin 200 mg/kg)

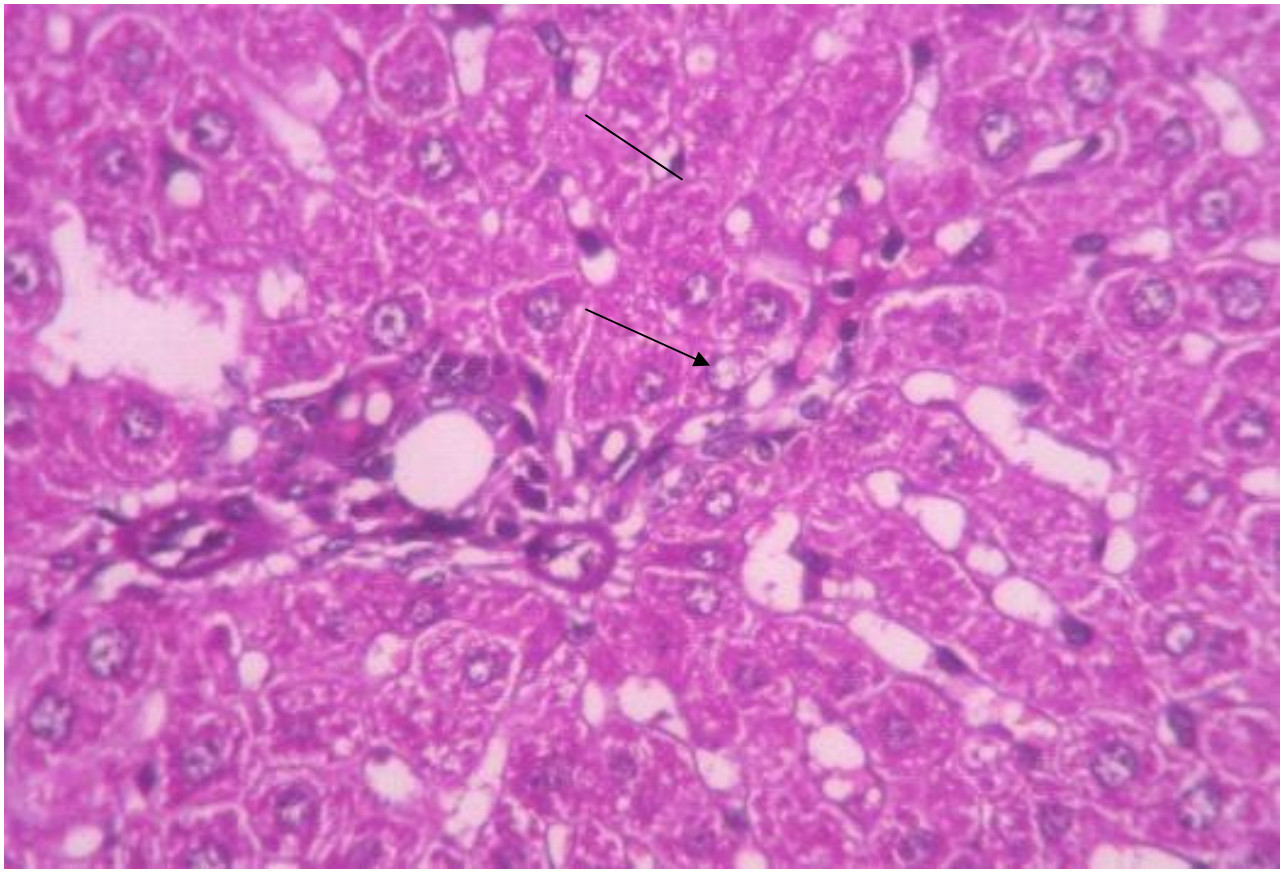


Fig 4 : AECC of 400 mg/kg shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells

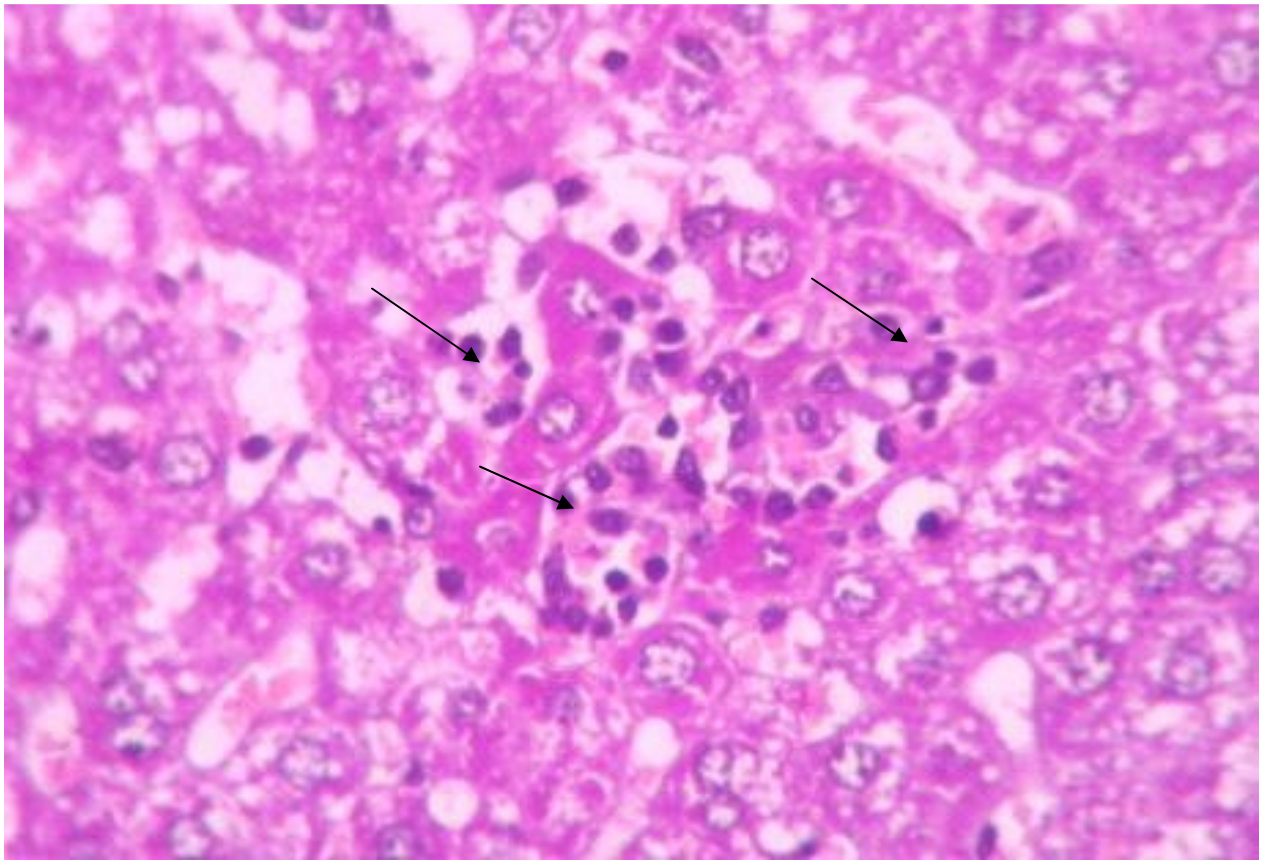
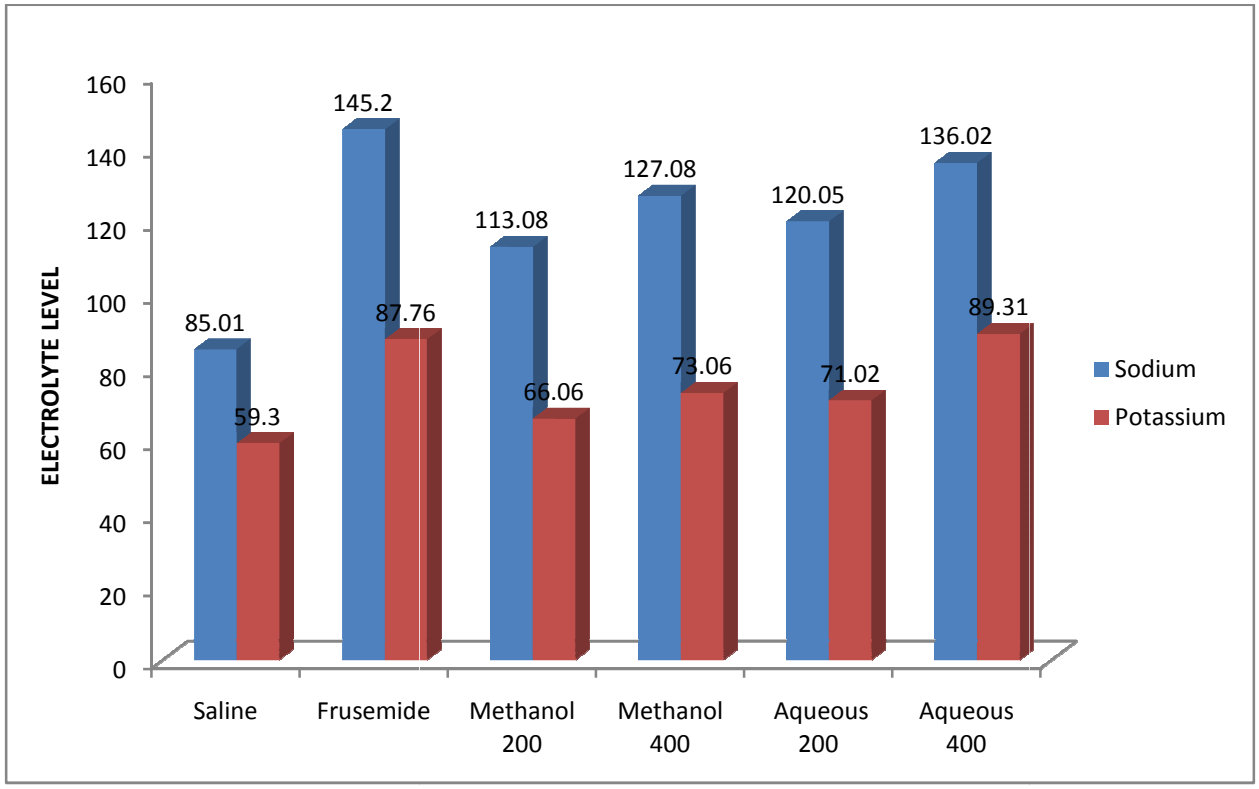


Fig 5 : MECC of 400 mg/ kg shows that regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells which is similar to silymarin treated group.

Effect of methanolic and aqueous extracts of *Canthium coromandelicum* and frusemide on sodium and potassium levels

Treatment	Dose mg/kg	Sodium (mg/kg body weight/day)	Potassium (mg/kg body weight/day)
Control(Normal saline)	10	85.01 ± 2.82	59.30 ± 1.32
Standard Drug (frusemide)	20	145.2 ± 2.07**	87.76 ± 1.782**
MECC	200	113.08 ± 2.04**	66.06 ± .0642*
MECC	400	127.08 ± 0.98**	73.06 ± 0.519**
AECC	200	120.05 ± 2.91**	71.02 ± 0.5033**
AECC	400	136.02 ± 1.22**	89.31 ± 0.290**

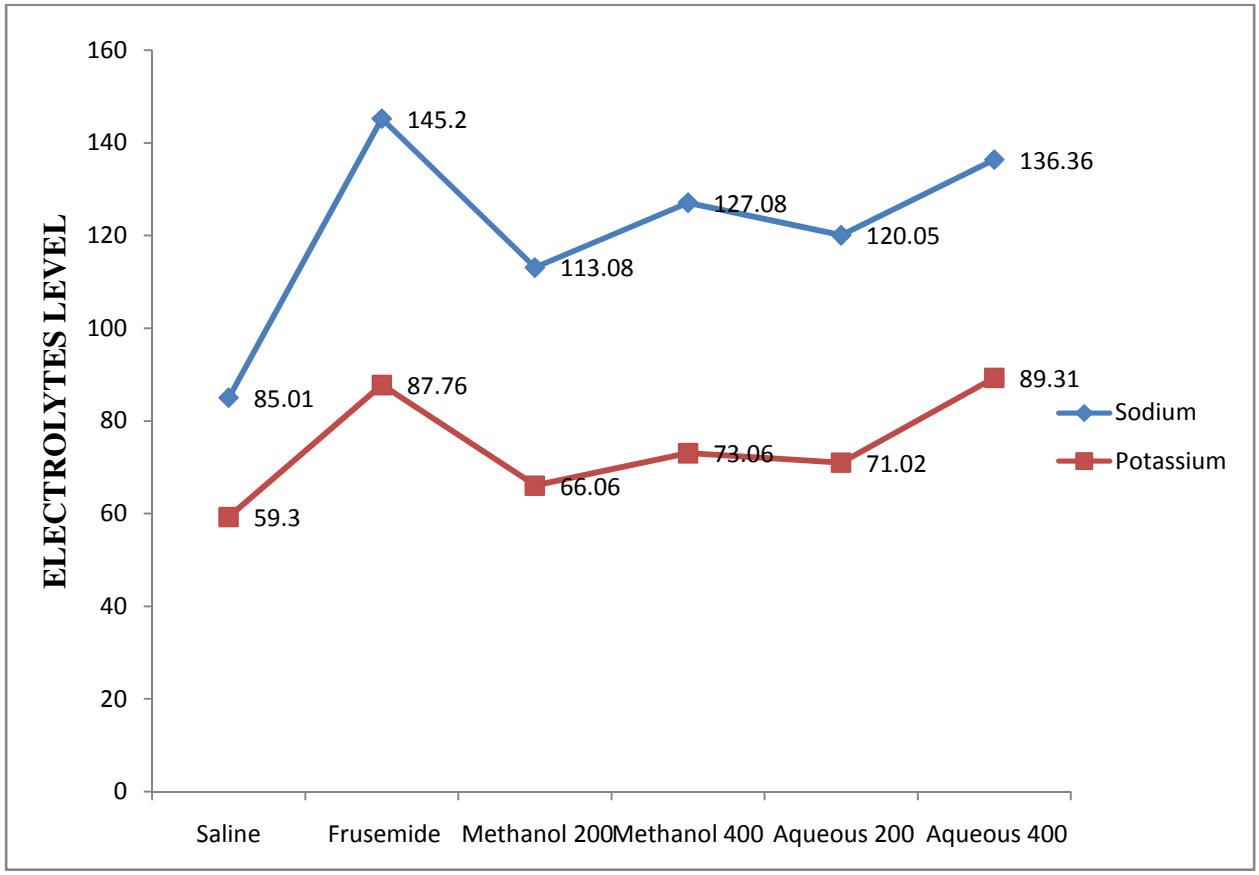
Effect of methanolic and aqueous extracts of *Canthium coromandelicum* and frusemide on sodium and potassium levels



The Various leaves extracts of *Canthium coromandelicum* and frusemide on urne level, diuretic index and pH

TREATMENT	DOSE (mg/kg)	URINE VOLUME (ml) mean ± SEM	DIURETIC INDEX	pH
Control(Normal saline)	10	4.57 ± 0.13	-	7.34 ± 0.18
Standard Drug (frusemide)	20	7.84 ± 0.18**	1.7155	7.04 ± 0.32
MECC	200	6.51 ± 0.18**	1.4245	7.32 ± 0.24
MECC	400	7.12 ± 0.12**	1.5579	7.22 ± 0.21
AECC	200	5.16 ± 0.13*	1.1291	6.88 ± 0.22
AECC	400	6.07 ± 0.13**	1.3282	6.05 ± 0.27

The Various leaves extracts of *Canthium coromandelicum* and frusemide on urne level, diuretic index and pH



HEPATOPROTECTIVE ACTIVITY :

In this study, the hepatoprotective action of *Canthium coromandelicum* was evaluated using Paracetamol induced hepatic injuries are commonly used for the screening of hepaprotective drugs and the extent of hepatic cells damaged was observed. The pathological changes such as elevated levels of SGOT,SGPT and hepatocyte damage was recovered by the methanolic and aqueous extract of the leaves of *Canthium coromandelicum*.

DIURETIC ACTIVITY :

In this study, the diuretic action of *Canthium coromandelicum* was evaluated using furosemide which is high-ceiling diuretic, under controlled laboratory conditions. As diuretic diuretic theraphy may lead to number of life threatening electrolytic disorders and toxicity so safety profile was carried out. The urine levels and electrolyte levels such as sodium and potassium levels from the methanolic and aqueous extract of the leaves of *Canthium coromandelicum* are similary to that of frusemide treated group of animals.

CONCLUSION

HEPATOPROTECTIVE ACTIVITY :

The hepatoprotective effect of aqueous and methanolic extract of *Canthium coromandelicum* leaves was confirmed by the following measures: The isolated livers from the toxicant (paracetamol) treated animals exhibited increase in wet liver weight. Indeed, extract treated animals exhibited decrease in the values of above physical parameters as an indication of hepatoprotection. Serum marker enzymes such as SGPT, SGOT and total bilirubin, showed marked increase. The same is observed in liver diseases in clinical practice and hence are having diagnostic importance in the assessment of liver function. In the present study, the methanolic and aqueous extract of *Canthium coromandelicum* leaves significantly reduced the elevated levels of above mentioned serum marker enzymes. Hence, at this point it is concluded that the methanolic and aqueous extract of *Canthium coromandelicum* leaves possesss hepatoprotective activity.

In support to this study, histopathological results also show significant activity of the plant. In toxicant treated animals there will be severe disturbances in the cytoarchitecture of the liver. The same is observed in case of humans who are suffering from major liver disorders. But in the methanolic and aqueous extract of *Canthium coromandelicum* leaves treated group animals exhibited minimal hepatic derangements and intact cytoarchitecture of the liver was maintained. In addition to this there is regeneration of hepatocytes also observed, which indicating hepatoprotective activity.

Finally based on improvement in serum marker enzyme levels, physical parameters, functional parameters and histopathological studies, it is concluded that the methanolic and aqueous extract of *Canthium coromandelicum* leaves possesses hepatoprotective activity and thus supports the traditional application of the same under the light of modern science.

DIURETIC ACTIVITY :

The diuretic effect of aqueous and methanolic extract of *Canthium coromandelicum* leaves was confirmed by the following measures: The urine levels and electrolyte levels such as sodium and potassium levels from the frusemide treated animals

exhibited increased in above levels. Indeed, the aqueous and methanolic extract of *Canthium coromandelicum* leaves extract treated animals exhibited the significantly same in urine levels and electrolyte levels. Hence, at this point it is concluded that the aqueous and methanolic extract of *Canthium coromandelicum* leaves possesss diuretic activity.

SUMMARY

The present study was aimed to assess the hepatoprotective activity and diuretic activity of aqueous and methanolic extract of *Canthium coromandelicum* leaves. LD50 studies were conducted in albino rats with aqueous and methanolic extract of *Canthium coromandelicum* leaves according to OECD guideline No.425 and was found safe upto the dose level of 4 gm/kg confirming its non-toxic nature.

HEPATOPROTECTIVE ACTIVITY

The hepatoprotective activity was studied in paracetamol induced hepatotoxic animal model. The Physical parameter wet liver weight , Biochemical parameters like serum SGPT, SGOT, and total bilirubin levels, and histopathology of livers were considered as major parameters of study.

Paracetomol induced hepatotoxicity was significantly prevented by pretreatment with aqueous and methanolic extract of *Canthium coromandelicum* leaves. Decrease in wet liver weight , reduction in elevated biochemical parameter levels like serum SGPT, SGOT, and total bilirubin, after treatment with aqueous and methanolic extract of *Canthium coromandelicum* leaves confirmed the hepatoprotective effect of extract under study. In liver injury models in rats restoration of hepatic cells with minor fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepatoprotection.

Based on improvement in serum marker enzyme levels, physical parameters, and histopathological studies it was concluded that aqueous and methanolic extract of *Canthium coromandelicum* leaves possesses significant hepatoprotective activity in the doses used.

The hepatoprotective activity was studied in paracetamol induced hepatotoxic animal model. The Physical parameter wet liver weight , Biochemical parameters like serum SGPT, SGOT, SALP, and total bilirubin levels, and histopathology of livers were considered as major parameters of study.

DIURETIC ACTIVITY

Frusemide induced diuresis was compared with aqueous and methanolic extract of *Canthium coromandelicum* leaves in which urine level increased and electrolyte levels such as sodium and potassium level excretion is significantly same as that of standard drug frusemide .after treatment with aqueous and methanolic extract of *Canthium coromandelicum* leaves confirmed the diuretic effect of extract under study.

Based on improvement in excretion of urine level, sodium and potassium level it was concluded that aqueous and methanolic extract of *Canthium coromandelicum* leaves possesses significant diuretic activity in the doses used.

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